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KIDNEY FUNCTION.[1]

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THE subject of this paper, kidney function, is far too broad for discussion within the permissible limits of one lecture. I therefore propose to restrict what I have to say to the subject:

Glomerular Function and the Modes of its Regulation.—What I have to present is based upon work which has been proceeding with interruptions in the laboratory of pharmacology of the University of Pennsylvania for a number of years past; this work has been jointly carried on by Dr. O. H. Plant and myself during the years 1915 to 1920 and by Dr. Carl F. Schmidt and myself from September of last year until now. Their collaboration has been invaluable.[2]

I. Evidence that the Glomerulus of the Kidney is the Chief Structure Concerned in the Renal Elimination of Fluid from the Blood.—Until the classical work of the English anatomist William Bowman, published in 1842, there was no convincing evidence that connection existed between the Malpighian bodies and the uriniferous tubules. By extraordinary skill in dissection, Bowman proved that the capsule of the Malpighian body is the expanded extension of the membrane of the tubule. His first identification of the complete unit of structure by which urine is formed must therefore be regarded as the beginning of modern study of renal function.

[1] A lecture delivered before the Harvey Society, New York, February 26, 1921.

[2] The results of this work will shortly be published in the American Journal of Physiology.

Appended to Bowman's very complete description of the vascular arrangements of the kidney is a theory of the parts played by tubule and glomerulus in the formation of urine. He laid emphasis upon the structural similarity of the epithelium of the tubules and that of secreting glands, and drew the inference that the tubules eliminate from the blood "the peculiar principles found in the urine." He laid equal emphasis upon the dissimilarity of structure of tubules and capsule, and stated his conception of the significance of this dissimilarity in these words, which, though frequently quoted, may well be repeated:[3]

"Thus the Malpighian bodies are as unlike as the tubes passing from them are like the membrane, which, in other glands, screens its several characteristic products from the blood. To these bodies, therefore, some other and distinct function is with the highest probability to be attributed. The peculiar arrangement of the vessels in the Malpighian tufts is clearly designed to produce a retardation in the flow of blood through them. . . . It would indeed be difficult to conceive a disposition of parts more calculated to favor the escape of water from the blood than that of the Malpighian body. A large artery breaks up in a very direct manner into a number of very minute branches, each of which suddenly opens into an assemblage of vessels of far greater aggregate capacity than itself, and from which there is but one narrow exit. Hence must arise a very abrupt retardation in the velocity of the current of blood. The vessels in which this delay occurs are uncovered by any structure. They lie bare in a cell from which there is but one outlet, the orifice of the tube. This orifice is encircled by cilia in active motion directing a current toward the tube. These exquisite organs must not only serve to carry forward the fluid already in the cell, and in which the vascular tuft is bathed, but must tend to remove pressure from the free surface of the vessels, and so to encourage the escape of their more fluid contents. Why is so wonderful an apparatus placed at the extremity of each uriniferous tubule if not to furnish water to aid in the separation and solution of the urinous products from the epithelium of the tube?"

This is the first suggestion, founded, it is true, upon teleological argument from structure, that the glomerulus is the chief site of fluid elimination in the kidney. This suggestion developed into universal belief. The experiment which established its truth was not made until 1878, when Nussbaum[4] performed the operation in frogs of ligation of the renal arteries. This excluded the glomeruli from the circulation, but, owing to the double blood supply of the frog's kidney, did not abolish circulation in the vessels of the tubules. The result of this ligation was cessation of urine elimination.

[3] Phil. Tr. Roy. Soc., 1842, pp. 74 and 75.
[4] Arch. f. d. ges. Physiol., 1878, 16, 139; 1878, 17, 580.

This observation, confirmed by others,[5] nearly approaches to direct proof of the assumption made by the older anatomists. Since it is completely in harmony with considerations of structure, since it is supported by a mass of less direct evidence obtained in other ways, and since there is no opposing evidence, so far as I am aware, we may regard this question, so fundamental to all further study of kidney function, as satisfactorily settled.

II. The Nature of the Process by which Fluid is Separated from the Blood in the Glomerulus.—In Bowman's statement of his hypothesis that the glomerulus separates water from the blood, no clear idea is given of the nature of the process. Unaware of the epithelium which covers the glomerular tuft, he regarded the capillaries as projecting naked into the capsule, and he speaks of the cilia at the orifice of the tubule as presumably having power of diminishing pressure on the capsular side of the capillary tuft and so facilitating escape of fluid from the blood. It seems to me that his words vaguely indicate the escape of fluid because of pressure within the capillary vessels.

There is no such vagueness, however, in Carl Ludwig's statement, made in 1844, of his conception of the process.[6] Using the anatomical facts demonstrated by Bowman and confirmed by himself, and applying principles of hydraulics, he stated that a significant pressure must be exerted by the blood within the glomerular capillaries upon their walls, and that this pressure must result in the filtration of a certain amount of fluid through them. He assumed that the membrane through which the fluid passed was normally impermeable to proteins, to fats and to salts which might be combined with these, and hence that the urine as formed in the glomerulus is a protein-free filtrate containing blood crystalloids in the proportion in which they exist free in the blood.

I have no wish to enter in great detail into a discussion of the evidence for and against the filtration theory; it has been adequately reviewed many times and forms part of current physiological teaching. Since in the development of what is to follow an appreciation of the chief elements of strength and of weakness in the filtration theory is necessary, I make no apology for briefly presenting the most important facts. The question whether the glomerulus filters fluid or secretes fluid is more than academic. A well-based conviction that the understandable process of filtration is the chief factor of glomerular activity permits clearly defined views concerning the nature of alterations in glomerular function which occur in health and disease. It carries with it as an inevitable corollary a conviction of reabsorption of both water and dissolved substances

[5] Adami: Jour. Physiol., 1885, 6, 582. Beddard: Ibid., 1902, 28, 20. Cullis:, Ibid., 1906, 34, 250. Bainbridge, Collins and Menzies: Proc. Roy. Soc. B., 1913, 86, 355.

[6] Wagner's Handwörterbuch der Physiologie, 1844, 2, 637.

from the lumen of the tubule, for no other process could account for the difference in composition between a blood filtrate and the urine as it leaves the kidney. Absence of such conviction, on the other hand, necessitates refuge in the conception of "secretion," a word implying ignorance or uncertainty of processes involved and an ill-defined point of attack on the further questions of alterations in renal function.

There are three groups of experiments which, I think, form the chief support of the filtration theory.

First, experiments which demonstrate the parallelism between urine elimination and renal blood-pressure. These include the experiments in Ludwig's laboratory by Goll,[7] showing that changes in general arterial blood-pressure, induced by vagus stimulation, hemorrhage, injection of blood or ligation of large arterial trunks caused changes in a similar sense in urine flow; and those by Hermann,[8] in which diminution in urine was found to follow partial obstruction of the renal artery. They include also numerous experiments which developed from Claude Bernard's discovery of vasomotor nerves, experiments in which the nerve supply of the kidney was either divided or stimulated, and resulting increase or decrease in urine found to be attributable to dilatation or constriction of the vessels in the kidney.[9] It was recognized by Ludwig and his colleagues that such changes in the renal circulation as were studied in these experiments involved alterations not only in renal blood-pressure but in velocity and volume of renal blood flow as well. Reasons were adduced (Hermann) for belief that the effective variable in these experiments was that of pressure. The force of the experiments and the influence of Ludwig were such that his conception of glomerular filtration and tubular reabsorption became the generally accepted view.[10]

The second group of experiments to which I refer is based upon this principle of physics: that in order to separate a dissolved substance from its solvent by filtration through a membrane, permeable by the solvent but not by the dissolved substance, filtration pressure must be greater than the osmotic pressure of the dissolved substance. Tammann,[11] of Rostock, in 1896 showed that the osmotic pressure of all the substances dissolved in the blood plasma was nearly eight atmospheres (5840 mm. Hg,); that the osmotic pressure of the organic solids of blood plasma amounted to 840 mm. Hg. (He regarded the osmotic pressure of proteins as negligible.)

[7] Ztschr. f. rat. Med., 1854, **4**, 78.

[8] Sitzungsber. d. kais., Akad. d. Wiss., Wien, 1862, **45**, **2**, 317.

[9] Bernard: Leçons sur les proprietes physiologiques des liquides de l'organisme, 1859, **2**, 169. Eckhard: Beitr. z. Anat. u. Physiol., 1869, **4**, 155. Ustimowitsch: Ber. u. d. Verh. d. k. Sachs. Gesell. d. Wiss. z. Leipsig (Math.-phys. Cl.), 1870, **22**, 430. Grützner: Arch. f. d. ges. Physiol., 1875, **11**, 370.

[10] Cf. Heidenhain: Hermann's Handbuch der Physiologie, **5**, **1**, p. 318.

[11] Tammann: Ztschr. f. physikal. Chem., 1896, **20**, 180.

Since no pressures of this order of magnitude are to be found in the animal circulation, he concluded that the only substances of plasma which could physically be held back in the glomerulus are the proteins; hence the fluid separated in the glomerulus must be the water of the blood containing all dissolved substances except proteins.

Starling,[12] in the same year, discovered that the osmotic pressure of plasma proteins amounted to from 30 to 40 mm. Hg. He showed that a force of this magnitude exerted by substances retained within the bloodvessels was sufficient to explain in part the absorption of fluid from tissue spaces into the bloodvessels. In 1899 he extended this reasoning to the explanation of glomerular function.[13] By improved method he redetermined the osmotic pressure of plasma protein and obtained the figure 25 to 30 mm. Hg. If the osmotic pressure of plasma protein is the force which blood-pressure must overcome in order to filter fluid from the blood in the glomerulus, then it should be found that the lowest arterial blood-pressure compatible with urine elimination should be slightly above this. His own experiments and those of many others showed that urine ceased to be eliminated when arterial pressure fell below 40 mm. Hg. Further, if glomerular function is filtration then the difference between arterial blood-pressure and the maximum pressure in the ureter against which urine can be eliminated should be almost that of the osmotic pressure of the proteins. He found this difference during profuse diuresis in the dog to be 32 to 43 mm. Hg. These results, confirmed and extended by Knowlton,[14] are so completely in accord with the demands of the filtration theory that they furnish the strongest support for it.

The third group of experiments in this connection are those of Barcroft and Straub[15] made in 1910. They applied to the kidney the methods so fruitfully developed by Barcroft for estimating the rate of metabolism of organs. Saline diuresis—*i. e.*, diuresis following injection of sodium chloride solutions—was found to be unaccompanied by increase in utilization of oxygen or formation of carbon dioxide. Knowlton and Silverman[16] later showed that this was true for diuresis following injection of pituitrin. The conclusion was drawn that physical factors rather than "vital" or "secretory" are concerned in this increase in kidney function, the inference being that filtration is increased.

These are the facts which to my mind most nearly constitute "proof" of the filtration idea: they are reinforced by considerations of the structure of the glomerulus and by observations in other directions; that the more rapidly urine is eliminated the more nearly it comes to resemble a filtrate from the blood; that the

[12] Jour. Physiol., 1896, **19**, 312.
[13] Ibid., 1899, **24**, 317.
[14] Ibid., 1911–12, **43**, 219.
[15] Ibid., 1910-11, **41**, 145.
[16] Am. Jour. Physiol., 1918, **47**, 1.

glomerular fluid is alkaline, as tested by intravital indicators; that the osmotic pressure and chloride content of the cortex more closely resemble that of the blood than does that of the medulla. This collection of facts led Bayliss to write, in 1915, "The evidence for this (glomerular filtration) is overwhelming;"[17] and Cushny, in his development of the "modern" theory of urine formation, to accept glomerular filtration as a fundamental truth.[18]

It is easy to develop conviction of the truth of filtration by study of the work to which I have alluded. It is not so easy to hold it after consideration of some of the questions which have been put to the filtration theory and have not found satisfactory answer.

Heidenhain, in 1874, began the publication of his work on the kidney, from which developed the so-called Bowman-Heidenhain theory. As is well known he injected indigo carmin into the circulation and failed to find traces of it in the capsule or any staining of glomerular structures by it. Since it was to be found in the lumen of the tubule, and since the tubular epithelium was stained by it, he was forced to conclude that it had been secreted by the tubules and had not been filtered by the glomerulus.[19] This observation led him to further results which obliged him to deny the filtration-reabsorption theory completely and to attribute urine formation to secretory processes in the epithelium of glomerulus as well as of tubule—*i. e.*, to processes not explainable by known physical or chemical laws.

Most of Heidenhain's contentions have since been successfully met by adherents of the filtration idea. Cushny's monograph contains an admirable exposition of this subject. One objection, however, seems to me to have been least satisfactorily answered, and it happens that this is the one to which Heidenhain himself attached the most weight. It concerns the effects of compression of the renal vein upon urine elimination. The following is a translation of his own words.[20]

"But if mechanical filtration does really occur, then elimination of water must always increase with the pressure. An old experiment shows that this is not so. For if the pressure in the glomeruli is increased by partial or complete occlusion of the renal vein an immediate diminution in urine occurs.

"This fact contradicts the pressure hypothesis in the most abrupt (schroffstem) manner. . . .

"If it is considered that increase in aortic pressure, if only a few millimeters, often causes a considerable increase in urine, and that after partial or complete occlusion of the renal vein a considerable rise of pressure within the glomerular vessels must occur, then it is

[17] Bayliss: Principles of General Physiology, 1915, p. 355.
[18] Cushny: The Secretion of Urine, 1917.
[19] Heidenhain: Arch. f. mik. Anat., 1874, **10**, 1.
[20] Heidenhain: Hermann's Handbuch der Physiologie, 1883, **5**, pt. 1, pp. 324, 325.

apparent that here is a phenomenon completely inexplainable by the filtration hypothesis."

Ludwig[21] was aware of this objection and had met it by demonstrating that complete obstruction of the renal vein in the living animal caused such swelling of the veins within the kidney that the tubules were compressed and their lumina obliterated. Obviously, no urine could issue from the kidney under these circumstances. It appears that Heidenhain[22] accepted Ludwig's demonstration of the effects of complete occlusion, but he did not regard it explanatory of the events which follow partial closure of the vein. Slight obstruction of a degree sufficient to lessen but not to suppress urine flow could not cause such lessening by engorgement of veins with resulting closure of tubules. The fact that urine continued to flow, though at a lower rate, indicated that the tubules were patent. Paneth's[23] later experiments, showing the possibility of diuresis by sodium nitrate during constriction of the renal vein, confirmed this conclusion. For this reason Heidenhain regarded the failure of slight compression of the renal vein to increase urine flow as the strongest argument against the filtration hypothesis, and it was this that led him to the belief that the velocity of blood flow through the glomerulus, rather than the pressure of blood within it, was the determining factor in the first formation of urine in the kidney.

In answer to this objection it was pointed out by Tammann[24] that if fluid is filtered from the blood in the glomerulus any stagnation of flow in the glomerulus, as by venous obstruction, would lead to rapid increase in osmotic resistance to filtration. It has not been shown that this factor can be so effective during partial occlusion of the vein as to more than compensate for the increased glomerular pressure. It has been suggested by De Souza[25] that blocking of the renal vein causes reflex constriction of the renal artery, but no evidence of this has been presented so far as I am aware. Consideration of these matters leads me to think that the argument against filtration, based upon the effects of obstruction of the renal vein, has not been adequately answered.

Another series of obstacles in the way of unreserved acceptance of the filtration hypothesis has arisen from the comparison of urine elimination with vascular conditions in the kidney, as shown by oncometer records of kidney volume, and these difficulties have increased with the later development of improved methods of estimation of the flow of blood through the kidney.

The oncometer, first applied to the study of renal physiology by Roy and Cohnheim in 1883, registers changes in the total volume of the kidney; these changes are commonly referred to alterations in

[21] Sitzungsber. d. k. Akad. d. Wiss. zu Wien, November, 1883.
[22] Hermann's Handbuch, 5, 1, p. 317. Cf. also Paneth, pp. 550, 551.
[23] Arch. f. d. ges. Physiol., 1886, 39, 515.
[24] Loc. cit.
[25] Jour. Physiol., 1900–01, 26, 139.

the state of the renal bloodvessels. In 1900 and 1901 Gottlieb and Magnus[26] made an admirable series of observations on blood-pressure, kidney volume and urine flow during diuresis. Following the injection of single doses of diuretics, remarkable parallelism between urine elimination and vascular dilatation, as shown by the oncometer, was observed; but when repeated dosage was given this parallelism failed. Diuresis was observed to increase in some instances at a time when renal vessels, as shown by the oncometer, were constricting; in others it diminished while renal vessels were similarly shown to be dilating.

These objections were materially supported by the late Professor Brodie, of Toronto. He extended the observations of Magnus and Gottlieb by including in his experiments direct estimations of blood flow through the kidney. In his lecture before the Harvey Society in 1910[27] and in his Croonian lecture of 1911[28] he stated that, following the injection of diuretics, in five experiments he had observed the following coincident phenomena: Increased kidney volume (indicative of dilatation of vessels); diminished blood flow (indicative of constriction of vessels); increased urine elimination.

Both Magnus and Brodie apparently accepted the common implication of vascular changes, namely, that dilatation of renal vessels means rise of intraglomerular pressure, and constriction of renal vessels means decrease in intraglomerular pressure; and hence their observations became so self-contradictory when viewed in the light of the filtration hypothesis that they were forced to abandon it.

In the considerations thus far advanced I have hoped to show that in spite of the array of strength back of the belief that urine is first formed in the glomerulus by a process of filtration, sound observations exist, made by most competent observers, which have forced them to deny it. Concern over these difficulties, and the necessity of a conviction concerning them, led to a series of experiments by my colleagues and myself which have, we think, a direct bearing on their solution.

In Hermann's[29] second paper on kidney function, published in 1862, it is stated that "The effect of pressure changes as compared with other factors which modify urine excretion can only be brought out clearly when one has control over the blood entering the vascular system and can regulate it at will." I cite this to show that the desire for some sort of artificial experimental control over circulatory conditions in the kidney in order to reduce the number of variables in an experiment is very old. Hermann devised a clamp by which the caliber of the renal artery could be narrowed, hoping to identify the effects of lowered renal blood-pressure by this means; somewhat

[26] Arch. f. exp. Path. u. Pharm., 1901, 45, 223.
[27] Harvey Lectures, 1909–10, 5th series, p. 81.
[28] Brodie: Proc. Roy. Soc., B., 1913–14, 87, 571.
[29] Loc. cit.

similar experiments have more recently been made. A defect in such experiments, recognized by their authors and emphasized by Heidenhain, is that such a device simultaneously alters both blood-pressure and blood flow in the renal circulation, and there is no direct means of distinguishing effects due to one of these to the exclusion of the other.

During the years 1912–14, C. K. Drinker and I designed and constructed an apparatus for the perfusion of isolated surviving organs capable of pumping a pulsating stream of fluid in a manner similar, as pulse records showed, to that of the heart.[30] Its volume output was controllable within fairly wide limits. With it we perfused the dog's kidney and were able to show that the fluid which issued from the ureter was urine and not a transudate. In 1914–15 Dr. O. H. Plant and I elaborated a method for perfusing the rabbit's kidney *in situ* with this apparatus.[31] Our method possessed these advantages and possibilities:

1. The perfusion fluid was the undiluted blood of the animal whose kidney was perfused plus blood taken fresh from another animal of the same species. Hirudin was used to prevent clotting.

2. The artificial circulation through the perfused kidney was inaugurated without any interruption in blood flow through the organ, and in some instances urine flow continued without interruption during the change from normal to the artificial circulation.

3. While the output of the perfusion apparatus could be varied at will, for any particular adjustment the output was constant, regardless of the resistance offered by the vessels through which it drove the blood. It was thus possible to alter pressure by various means within the kidney vessels without simultaneous alterations in volume flow or velocity of blood in them. It is in this respect that our experiments differed essentially from those of earlier workers.[32]

The means which we used to alter pressure in the circulation of the perfused kidney were these: stimulation of the splanchnic nerve; injection of adrenalin; partial occlusion of the renal vein.

Since all of these agencies raised pressure in the renal circulation, and since the conditions of our experiment were such that they could not materially change the blood flow, we seem justified in attributing such results as were obtained to changes in renal blood-pressure.

Each of the three agencies tested increased urine formation in a number of experiments practically without exception.

It will be noted that among these agencies employed is venous obstruction, which in the intact animal always causes diminution of urine, a fact regarded by Heidenhain as the strongest argument

[30] Richards and Drinker: Jour. Pharm. and Exper. Ther., 1915, **7**, 467.
[31] Richards and Plant: Ibid., p. 485.
[32] Richards and Plant: Am. Jour. Physiol., 1917, **42**, 592.

against filtration. In our experiments, in which it increased urine, there was no stagnation of blood in the glomerular capillaries which might neutralize the effects of increased glomerular pressure as a filtering force. The experiment seems to me to remove the force of Heidenhain's objection and to confirm the suggestion that in the intact animal partial occlusion of the renal vein so lessens the rapidity of renewal of blood in contact with the glomerular endothelium that the effect of increased filtering forces is nullified.

III. Regulation of Glomerular Pressure.—The experiments just described have served us in three ways: they yield evidence that rise of pressure alone in the renal circulation can cause increase in urine; they point to a solution of Heidenhain's difficulty which is consistent with the filtration theory; and they provide a point of departure for an analysis of the effects of increased renal pressure. In this last connection the action of adrenalin has been most useful.

If the vascular reaction of a perfused kidney to minute doses of adrenalin is compared with the same reaction of another structure similarly perfused—*e. g.*, the leg—a striking difference appears. Any dosage of adrenalin which causes constriction of the vessels of the leg also causes diminution in volume of the leg, as shown by the oncometer. When a perfused kidney is similarly tested it is found that large dosage of adrenalin causes a similar effect, *i. e.*, constriction of vessels as shown by rise of perfusion pressure and shrinkage of volume of the kidney; but smaller doses, which still cause some degree of constriction of vessels, as shown by the perfusion pressure, cause either no change in or distinct swelling of kidney volume.

In this experiment with the kidney we have an apparent paradox of coincident constriction of vessels, as shown by rise of perfusion pressure, and dilatation of vessels, as shown by swelling of the kidney.

The only reasonable explanation of this paradox which has occurred to us is this: between the afferent and efferent vessels of the glomerulus is interpolated the distensible capillary area of the glomerular tuft. The walls of the afferent and efferent vessels both contain smooth muscle; both are supplied with nerve fibrils (presumably sympathetic), ending in contact with the muscle cells.[23] If under the conditions of our experiment the efferent vessel were constricted a passive rise of pressure must occur in the glomerular capillaries proximal to it, and the distention of these, thus brought about, might cause swelling of the kidney. In support of this view we recall the generalization which Elliott's work has established, namely, that the action of adrenalin is equivalent to stimulation of sympathetic innervation: and we have an observation of our own, made with the frog's kidney, which shows unmistakably that adrenalin has the power of constricting bloodvessels peripheral to the glomerulus.

[23] Smirnow: Anat. Anzeiger, 1901, **19**, 347.

In the minds of many physiologists a certain stigma appears to attach to perfusion experiments with isolated organs when the attempt is made to apply results so obtained to the interpretation of events within the intact animal body. If it were possible to demonstrate constriction of the efferent vessel by adrenalin in the intact animal with associated diuresis the force and usefulness of the experiments just cited would be increased. Reason for thinking that this might be possible seemed to exist. The efferent vessel of the glomerulus is a narrower tube than the afferent vessel. A constrictor influence, acting alike on both, would therefore produce a greater increase in frictional resistance to blood flow in the smaller (efferent) vessel; for this reason it seemed probable that very minute amounts of constrictor substances might, by more effective constriction of the efferent vessel, produce simultaneously three effects in the intact animal: diminution in blood flow through the kidney by constriction of efferent vessel; increase in urine by increased glomerular pressure; swelling of the kidney by distention of the glomerular capillaries. The event showed that this combination could be demonstrated following minute, but clearly constrictor, doses of adrenalin and pituitrin.

These experiments seem to me to yield evidence not only that the glomerular process is filtration but also that intraglomerular pressure—filtration pressure—is regulated by the relative degree of constriction or dilatation of the afferent and efferent vessels. This latter belief is intimated in a statement by Ludwig[34] in 1856:

"Since the afferent and efferent vessels of the glomerulus, as well as the roots of the renal vein, contain muscle within their walls, the possibility exists that the blood stream in the kidney changes according to the contractions of these muscles, even though the movements of the heart and the general circulation in the organism remain unchanged."

In connection with the action of constrictor substances, like adrenalin and pituitrin, it becomes apparent how it is possible for the same substances in different dosage to produce opposite effects. Minute amounts causing more effective constriction of the smaller efferent vessel may increase urine by increasing intraglomerular pressure; larger amounts, by constricting both afferent and efferent vessels, may diminish urine by decreasing ingress to the glomerulus and so lessening both intraglomerular pressure and velocity of flow. The literature shows that small dosage of adrenalin may cause diuresis. Nothing is easier to determine than that larger doses cause partial or complete suppression of urine. Similar apparently contradictory evidence concerning pituitrin can be found.

Permit me now to revert to Brodie's statement of a group of occurrences which he regarded as completely inconsistent with the

[34] Lehrbuch der Physiologie des Menschen, 1856, 11, 257.

filtration theory: diminished blood flow through the kidney, increase of kidney volume and increase in urine. This group of occurrences, as we think our experiments indicate, is precisely that which would be expected as a result of preferential slight constriction of the efferent vessel, and instead of being inconsistent with the filtration theory is demanded by it.

From this reasoning it would seem as though any substance which is constrictor to renal vessels should, in suitable high dilution, show evidence of diuretic power unless it lowers general blood-pressure or diminishes permeability of the glomerular membranes. This supposition is now being tested.

I am inclined also to suggest another generalization, based upon this evidence that intraglomerular pressure may be altered by more effective action of a substance upon the efferent than upon the afferent vessel. It concerns the action of arterial dilator substances in general. If it be agreed that glomerular urine is a protein-free filtrate from blood, then it follows that any substance in solution in the blood which causes dilatation of renal arterioles, and which in part passes out of the blood with the glomerular filtrate, must from these facts be potentially diuretic; for its effective concentration will be greater in the blood in the afferent vessel than in the blood in the efferent vessel; and its dilator action will be greater on the afferent than on the efferent vessel, and for this reason alone intraglomerular pressure must rise.

It should be noted that by effective concentration I mean concentration in relation to the colloids of the blood.

Without having the direct evidence to support this idea, I venture to present it in the belief that it is applicable to the glomerular behavior of a large number of substances—water, urea, the caffein series, salts, etc., all of which are vasodilator, and all of which, we believe, leave the blood stream as it passes through the glomerulus.

If we regard the renal arterioles as very sensitive both to constrictor and dilator substances, as they most certainly are, and if we admit the possibility of such preferential action as I have indicated on either efferent or afferent vessel, we must see in this a very delicate mechanism whereby intraglomerular pressure, and hence the first formation of urine is regulated in accordance with the chemical composition of the blood.

IV. Description of Glomerular Circulation.—In this description of experiments, both of our own and of others which concern the nature of glomerular function and its mode of regulation, an implication has been permitted which now appears to me to require correction. Its correction does not, I think, materially alter the force of the conclusions which have just been drawn, but it adds an element in the conception of glomerular regulation which may be of greater importance.

The kidney contains a great number of urine-forming units. The

number of glomeruli in the cat's kidney has been estimated at 16,000; in the kidney of a dog weighing 11 kilos, 150,000; in the human kidney, 2,000,000.[35] For each glomerulus there is a tubule. It seems to me that it has been tacitly assumed in the great bulk of writing on kidney function that the circulation through all of these units is at least roughly uniform—that they take equal part in the sum of activities which made up the total function of the whole organ. I have found one explicit statement of another conception: In Heidenhain's first work (1859) on kidney function he noted that the two kidneys may eliminate different amounts of urine, and he stated that it was simplest to assume that all parts of the kidney do not act to the same degree all of the time—that one part of the excreting surface may rest or be active while another part is in reserve.[36]

We have observations which indicate that this is a true conception.

In the development of the idea that glomerular pressure may be regulated by the degree of constriction of its efferent as compared with its afferent vessel, it became highly desirable that we get evidence as direct as possible concerning changes in size of the glomerulus during the action of adrenalin under controlled conditions of blood flow. In 1919 Krogh, of Copenhagen, published his extremely important paper on the behavior of capillaries in muscle.[37] He used methods of direct microscopical observation of muscles illuminated either by transmitted or reflected light. It occurred to us that the use of the same method might enable us to see the glomeruli of the kidney in operation, provided we could find a kidney sufficiently translucent to permit a certain penetration of light rays. In September of 1920 Dr. Schmidt and I found that the frog's kidney fulfills this demand. When we focused the light of an arc lamp or a 1000 watt "Mazda" lamp upon the ventral surface of an exposed frog's kidney *in situ*, the low power of the microscope showed in the interstices of the radicals of the renal veins nests of capillaries which to our view and that of competent microscopists could be nothing else than glomerular tufts. It soon became possible to distinguish the outline of the capsule, in many cases the entrance of the afferent vessel, and in not a few instances the exit of the efferent vessel as well. Because not all of these structures had the same appearance and to prevent an egregious error, we spent some time in attempts to identify a certain group of glomeruli in the living kidney, to follow this group through processes of fixation and embedding in order to prepare a stained section which should contain the structures examined in the living kidney. With the aid of Dr. B. Lucke this was successfully done. This identifica-

[35] Cited from Cushny: The Secretion of Urine, p. 5.

[36] Sitzungsberichte d. k. Akad. d. Wiss. zu Wien, Math.-Naturwiss. Cl., 1859, 36, 349.

[37] Jour. Physiol., 1919, 52, 409.

tion has given a sense of security concerning previous and subsequent observations which we might not otherwise have had.

When the lateral border of the ventral surface of the frog's kidney is observed in this way, the large renal veins and their tributaries are most prominent. Arterial branchings and the divisions of the renal portal vein, being deeper in the kidney, are less obvious. Details of the tubules are commonly indistinct. In the interstices of the veins are seen the glomerular tufts. They vary in size from 80 to 250 microns in diameter. Some show a great multiplicity of tortuous narrow channels, each of a diameter sufficient to permit passage of one red cell. The capillary wall is not easily seen. The blood flow through these channels is oftentimes bewilderingly rapid. In other instances the appearance is of another type: instead of a multiplicity of channels only one or two capillary loops are visible; these have wider diameter and show sluggish flow of more densely packed corpuscles. In the more slowly flowing blood streams pulsations are apparent; in the more rapidly flowing stream it may not be. Between these two extremes intermediate variations occur.

Concerning the problem which was the impetus for beginning these observations—*i. e.*, the question whether the glomeruli could be seen to swell as a result of the constrictor action of minute amounts of adrenalin upon the efferent vessel—the answer is still indeterminate. A series of measurements by Dr. Schmidt of glomerular diameters before, during and after the injection of doses of adrenalin of the order of 0.1 cc of 1 to 1,000,000 showed swelling, and in so far were confirmatory of our conclusions drawn from the mammalian experiments. But since a distinct improvement in the general circulation resulted from this injection the increased glomerular size may have been due to this. Before a final answer can be obtained the experiment must be performed on the kidney, perfused with blood at a constant rate. This experiment has not yet been made. Other features of the glomerular circulation seemed to demand more immediate study.

In our earliest experiments the variability of our preparations was striking. In some preparations as many as eight glomeruli could be counted in a field of 2 mm. diameter; in others only three or four in the whole kidney, in so far as it was accessible to inspection. Our frogs were pithed, and if two or three drops of blood were lost in this operation the number of glomeruli to be found in the kidney was small. If, however, such a frog were immersed in a saline bath, or if his abdominal cavity were filled with isotonic salt solution, the number of visibly active glomeruli increased.

Acting on the suggestion which this fact afforded, we have made a series of counts of the glomeruli which show active circulation under varied conditions.

Bits of silk thread were laid transversely across the surface of the kidney at approximately equal distances of about 2 mm.

(the diameter of our low-power field). From five to eight fields were thus separated for ease in counting. A bit of cover-slip was lightly laid over these, both to prevent displacement of threads and to avoid surface glare.

I shall cite figures to show alterations in the number of glomeruli in which blood was flowing before, during and after the introduction of various substances to be mentioned.

1. *Isotonic Salt Solution.*—As has been mentioned, salt solution is absorbed from the open abdominal cavity of the frog, and as a result circulation improves if it has been lessened from hemorrhage. These figures illustrate: Soon after preparation five fields showed 5 active, 8 inactive, total 13 glomeruli. Thirty minutes after salt solution had been introduced into the belly the same fields showed 28 active, 0 inactive, total 28 glomeruli. (By "active" glomeruli we mean those showing active circulation.)

2. *Injection of Blood.*—0.5 cc of whole blood was taken from the aorta of one frog and immediately injected into the anterior abdominal vein of a frog, whose glomeruli had been counted. Before injection, active glomeruli 10, inactive 27, total 37; five minutes after injection, active glomeruli 39, inactive 9, total 48.

3. *Injection of Isotonic Salt Solution.*—0.5 cc of 0.6 per cent. Before, 41 active, 9 inactive, total 50; ten minutes later, 54 active, 8 inactive, total 62; twenty minutes later, 44 active, 6 inactive, total 50.

4. Then *urea* 0.1 cc 20 per cent. After, 65 active, 2 inactive, total 67.

5. *Caffein,* 7 fields counted: Before injection, 81 active, 11 inactive, total 92; 0.1 cc 2 per cent *caffein,* five minutes later, 104 active, 0 inactive, total 104.

6. *Glucose.*—0.1 cc, 10 per cent glucose: Before injection, 31 active, 12 inactive, total 43. Followed by progressive increase in number of active until thirty-five minutes after, 62 active, 3 inactive, total 65.

7. *Hypertonic Sodium Sulphate* (5 per cent): 0.1 cc. Before, 6 active, 0 inactive, total 6; after thirteen minutes, 51 active, 0 inactive, total 51.

8. *Adrenalin* constrictor dose 0.1 cc, 1 to 100,000: 3 fields; before injection, 49 active; immediately after, 12 active; seven minutes later, 48 active.

9. *Pituitrin:* Constrictor dose, 0.1 cc 1 to 10 dilution of pituitrin "S." Before, 14 active, 5 inactive, total 19; after, 0 active, 16 inactive, total 16; later, 5 active, 12 inactive, total 17.

These and many similar observations have led to the conclusion that even under the most favorable of operative conditions, *i. e.,* with the least loss of blood, all the glomeruli of the kidney of the frog do not receive blood simultaneously. Conditions which depress the circulation, such as blood loss or destruction of the cord, or

agencies which constrict bloodvessels in the kidney, such as constrictor doses of adrenalin or pituitrin, lessen the number of glomeruli which receive blood. Plethora, absorption or injection of isotonic salt solution, hypertonic NaCl, hypertonic sodium sulphate, urea, glucose and caffein—all are capable of impressively increasing the number of glomeruli which receive blood at one time.

Not only is the number of glomeruli showing active circulation altered by the agencies which I have mentioned, but also the number of capillary loops within a single glomerulus which take part in the capillary blood flow. Earlier in this section I referred to glomeruli of two rather widely different aspects in so far as blood flow through them is concerned; one in which narrow rapidly flowing currents of blood indicate a complex network of tortuous channels; others in which one or two loops only are visibly filled with blood and in these blood usually flows more slowly and in a wider stream. The dilator agencies, urea, caffein, etc., mentioned above have the power of transforming a glomerulus of the latter type into one of the former.

Adrenalin, on the other hand, in constrictor dosage transforms a glomerulus showing a multiplicity of channels with rapid flow into one with fewer patent capillary loops and slow flow.

This indicates that just as all glomeruli in a kidney do not receive blood at once, so too in a single glomerulus not all the capillary loops need be patent at one time. Dilator (diuretic) agencies increase the number of capillaries in the glomerulus through which blood is flowing; constrictor substances and depression of the general circulation lessen the number.

Another characteristic of glomerular blood flow in the frog's kidney is that it is not always continuous but may be intermittent. Intermittence of glomerular flow is more liable to occur in a kidney showing active rapid circulation than in one in which blood flow is more sluggish. It was first observed by us in frogs after improvement of the circulation following absorption of salt solution; it has, however, been observed in frogs subjected to no other preparation than that required for looking at the kidney. The intermittence of blood flow may be of different types; in some instances there may be diminution in all and cessation in many at the same time, as though resulting from an influence outside of the kidney, as, for example, by changes in the general circulation or as a result of nervous stimuli to the bloodvessels. This type is easily understandable.

What we think of, however, as true intermittence is less easy to comprehend: two adjacent glomeruli may be situated within a few microns of each other; blood flow in one may stop completely, to be resumed after an interval, without interruption or even perceptible alteration in flow in the other. This phenomenon may be multiplied so that in a favorable field one sees a lively series of irregular interruptions in flow through the various glomeruli visible.

The interruptions bear no relation to the heart-beat. The intermittence of one glomerulus was timed with a stop-watch—15 seconds on; 12 seconds off; 27 seconds on; 11 seconds off.

In another, 103 seconds on; 10 seconds off; 90 seconds on; 45 seconds off.

In another preparation, 5 glomeruli were watched at once: Nos. 3, 4 and 5 stopped at the same time; Nos. 1 and 2 kept on actively. After three minutes No. 4 begins; Nos. 3 and 5 are blank.

We have tried to get a graphic representation of this phenomenon. A keyboard with five keys was connected each with a signal magnet arranged to write on a drum. Five glomeruli in a field were chosen and a key assigned to each. Discontinuance of flow was registered by pressing the key and keeping down until the flow resumed. The recorder also noted on the drum obvious variations in rapidity of flow which could not be designated as complete cessation or resumption. Charts were then made of these records.

Study of these charts forced the conclusion that while at times there are interruptions common to all, in the main the circulatory activity of one is independent of others; and the inference is drawn that a local regulatory mechanism must exist analogous to that shown by Krogh to exist in muscles.

Preliminary attempts to gain deeper insight into the circumstances of this phenomenon have been made. Not very much can safely be stated at present. We are sure that the phenomenon of intermittence persists after complete destruction of the whole central nervous system—brain and cord. There is evidence that intermittence of the glomerular circulation is commonly associated with simultaneous and synchronous intermittence of the afferent vessel. In this connection it is very striking that when flow stops abruptly in a single active glomerulus, corpuscles do not remain stagnant in its capillaries; they may remain for an instant, then they fade out of view and the whole glomerulus may become invisible. This must mean that the capillaries of the glomerulus possess power of independent contraction capable of emptying their lumina after blood has ceased to flow. This statement must be held as applicable to the afferent and efferent vessels, since they as well as the glomeruli are emptied when blood flow stops. Dr. Schmidt has made one observation which we hope to repeat: In one instance capillary flow in the glomerulus ceased abruptly and blood cells disappeared from it; but the afferent vessel remained full of blood, the corpuscles oscillating back and forth at the entrance of the glomerulus until presently the glomerular capillaries opened and flow through the whole structure resumed.

This emptying of the capillaries after cessation of flow—indicating, as we believe, independence of contractility of their walls—is much less marked or may be absent in the dilated sluggishly flowing capillaries of some of the glomeruli to which reference has been

made. These are likely to remain engorged with cells when the flow ceases. We take this to mean that there are normal differences in tonus and normal variations in tonus.

We do not yet know what the nature of the influence which regulate this tonus is.

Making the assumption that these observations are applicable to the mammalian kidney, they give us a conception of glomerular circulation different from that which I had previously held. Instead of a uniform circulation of blood through all the glomeruli, varying with general and renal blood-pressure, we conceive of a circulation, restricted under conditions of moderate blood flow to only a fraction of the glomeruli; and instead of equal circulation through all of the capillaries of a single glomerulus, we conceive of the possibility of restriction of flow through a fraction of the available pathway. This restriction in number of functioning glomeruli and in patent capillary loops may be brought about by general influences (circulatory and nervous) brought to bear from outside the kidney and unequally effective in different units of the kidney through anatomical differences, such as length of vessel; in addition we think of the restriction as due to a local, peripheral control of contractile power, not only of afferent and efferent vessels, but of the intervening capillaries as well. The phenomenon of intermittence permits us to think of alternating rest and activity of glomerular structures and prevention of damage such as would conceivably result from prolonged interruption of blood flow.

The phrases "dilatation of the kidney vessels" and "constriction of the kidney vessels" come to mean not only the increase and decrease in volume and rate of a stream already flowing, but also the increase and decrease in actual number of functioning glomeruli and of open glomerular capillaries. The possibilities in the direction of increase or restriction of filtering surface become more impressive.

On this basis it is not difficult to understand how relatively enormous changes can take place in glomerular blood flow without correspondingly great changes in the size of the kidney as registered by the oncometer, for obviously the capsule does not collapse when flow through the tuft ceases. It is easy to understand and to accept such puzzling experiments as those of Loewi—in which the ability of blood flow to increase under the influence of caffein in a kidney embedded in plaster of Paris was demonstrated.

It becomes easier to understand how a kidney might eliminate from blood of the same composition and in equal spaces of time urine of widely different composition, for a urine issuing as the result of highly active blood flow and high glomerular pressure in a smaller number of glomeruli must be different from that which issues as the result of slower blood flow and lower glomerular pressure from a larger number of glomeruli. The resorptive powers of the tubules would be effective to different degrees.

The difficulty of injecting the glomeruli *uniformly* even in fresh kidneys is comprehensible, as is also the lack of uniformity among the glomeruli in the action of circulating toxic substances.

A lead may be given concerning the causation of albuminuria under conditions not far removed from the physiological; it is a very old observation that complete arterial interruption of the circulation in the kidney for a short time is followed by albuminuria. If intermittence of glomerular flow is a normal phenomenon it would appear that albuminuria might occur if for any reason the duration of the normal intermittent cessation of flow increased.

I will give the briefest résumé of the chief points presented:

1. New evidence has been secured that increment of blood-pressure, uncomplicated by increment in velocity or volume of blood flow in the kidney, increases urine formation. This is regarded as added support of the filtration hypothesis.

2. Evidence has been secured indicating that some of the most weighty objections to the filtration hypothesis can be reasonably explained in a manner consistent with it.

3. Indications have been shown that nervous stimuli and chemical substances may exert different degrees of effective influence upon the afferent and efferent vessels of the glomerulus and that this may be a factor in that automatic regulatory control of glomerular filtration which is responsible in part for the maintenance of constancy of blood composition.

4. And, finally, a new description of the mode of circulation through the glomerular vessels has been presented which, when verified and extended, we hope will be of service in the study of the normal and pathological physiology of the kidney.

A FURTHER STUDY OF THE QUANTITATIVE VARIATIONS IN THE VIBRATION SENSATION.[1]

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THE first contribution to the study of the vibration sensation was made by Rumpf[2] in 1889. A steel tuning-fork was used, and the handle was so made that it was suitable for application of it to the skin surface after the vibration was started. Forks of different rate of vibration were used in this study, as it was found that in different parts of the body different rates of vibration were

[1] Read before the Association of American Physicians, May 11, 1921.
[2] Neurol. Centralbl., 1889, 8, 185.